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THE PRODUCTION OF INTERMEDIATE-WINGED APHIDS WITH SPECIAL REFERENCE TO THE PROBLEM OF EMBRYONIC DETERMINATION¹

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Aphids which are intermediate in structure between the parthenogenetic and the gametic type have already been studied in relation to the order of embryonic segregation (Shull, 1930, 1931, 1933). More abundant and more easily produced at will are those intermediates which are structurally between the winged and wingless parthenogenetic types. They offer the same opportunity to examine certain features of the mechanism of development as do any other intermediates which can be artificially produced.

Partly to obtain large numbers of these winged-wingless intermediates for analysis, and partly in the hope that the method of obtaining them would throw some light on their nature, extensive experiments in which the environmental agents known or believed to modify the aphid cycle were combined in a variety of ways were carried out over a period of years. This paper describes the experiments, which involved a total of 530,133 aphids, of which 9,152 were intermediate-winged.

The Stocks of Aphids

Two independent strains of aphids of the species *Macrosiphum solanifolii* have been used. One was collected near Ann Arbor in the year 1923 and has been maintained parthenogenetically ever since. This strain was clone A of a former paper (Shull, 1932) until it experienced a "mutation" in the fall of 1929, in which its characteristics were greatly altered, becoming clone A' of that paper. The other strain was collected in Ann Arbor in 1931; to date none of the results from this line has been published.

¹ Contribution from the Department of Zoölogy, University of Michigan.

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Since it has been shown that conditions affecting the aphids at least a generation in advance of the beginning of an experiment may modify the results of the experiment (Shull, 1935), four different stocks of each of the two strains were continuously reared. One was kept at a temperature of 24°, one at 14°, one at alternating temperatures (24° for 8 hours, 14° for 16 hours daily), and one at room temperature which fluctuated considerably. All were in continuous light which was not necessarily of uniform intensity.

The Method of the Experiments

In general a complete set of experiments would proceed as follows. From each of the eight stocks mentioned above a number of aphids, both winged and wingless, were drawn shortly before (or in a few cases just after) they became adult. Some of each type of female were placed at a temperature of 24°, others at 14°. In each of these temperatures some were kept in continuous light, others in intermittent light (8 hours light, 16 hours darkness). In addition, some aphids from each of the stocks except the one in room conditions were brought to room temperature for one generation prior to the beginning of the experiment; their offspring, having grown up at room temperature, were then reared in each of the various combinations of conditions just outlined.

It was never possible to do all of these things at once. However, all experiments involving one stock, and sometimes those from two of the stocks, could be done simultaneously. Since fairly consistent results were obtained from each treatment, it is unlikely that any serious error has been introduced by performing the various parts of an experiment at different times.

Effect of Continuous and Intermittent Light in the 1923 Strain

The results of these experiments can be presented only as total numbers of individuals of the several kinds produced under each combination of conditions. This is best done in tabular form. In branching tables, the contrasting conditions placed nearest the data are the ones whose effect is best shown, since pairs of adjoining numbers represent the contrast. Other contrasts are between numbers at some distance from one another, and are less readily seen. It will be necessary, therefore, to arrange the data in several ways to show the significant results.

In Table I the last column preceding the data (the fourth column) includes the two contrasted light conditions, namely, continuous and intermittent (8 hours of light, 16 hours of darkness). It is therefore the effect of light which is best shown by this table. To save space the

TABLE I

The percentages of winged and intermediate-winged offspring produced by both wingless and winged aphids of the 1923 strain in various combinations of light and temperature both during and before the experiments.

Stock parents came from	Temperature of parents preceding experiment	Temperature of parents during experiment	Light on parents during experiment	Offspring					
				From wingless parents			From winged parents		
				Total number of off-spring	Percentage winged	Percentage intermediate-winged	Total number of off-spring	Percentage winged	Percentage intermediate-winged
24°	24°	24°	Cont. 8-16	7,640 5,832	50.7 32.5	0.26 3.23	12,816 6,777	59.7 37.0	0.37 4.01
		14°	Cont. 8-16	7,835 5,592	46.5 16.6	0.18 5.17	17,056 17,335	42.4 19.2	0.28 6.35
	Room	24°	Cont. 8-16	2,522 1,650	47.4 38.4	0.12 3.03	2,261 1,737	40.0 28.6	0.04 4.38
		14°	Cont. 8-16	2,387 2,445	62.7 15.4	0.08 4.05	2,301 1,647	32.2 9.0	0.22 10.75
14°	14°	24°	Cont. 8-16	6,550 5,240	49.4 47.4	0.11 5.42	8,309 7,085	33.6 20.5	0.13 2.87
		14°	Cont. 8-16	7,182 5,307	55.0 20.4	0.07 8.95	6,806 4,084	21.3 11.5	0.13 11.75
	Room	24°	Cont. 8-16	880 846	51.1 39.7	0.11 5.52	3,329 3,326	42.4 29.8	0.24 2.56
		14°	Cont. 8-16	1,238 1,017	50.8 18.6	0.00 7.37	3,630 3,169	29.6 10.4	0.08 9.09
Alt.	Alt.	24°	Cont. 8-16	5,581 5,547	49.3 39.5	0.13 2.94	10,505 9,205	55.3 43.7	0.39 4.20
		14°	Cont. 8-16	7,257 5,702	35.8 13.0	0.12 7.87	12,786 8,975	39.6 12.9	0.13 10.92
	Room	24°	Cont. 8-16	2,902 2,709	44.9 35.4	0.21 3.03	4,094 3,545	35.8 25.3	0.07 2.03
		14°	Cont. 8-16	3,225 2,760	44.6 17.8	0.09 8.48	4,650 3,190	34.1 7.4	0.04 7.62
Room	Room	24°	Cont. 8-16	4,415 3,912	46.8 41.1	0.18 2.94	8,015 6,821	45.8 30.3	0.29 4.31
		14°	Cont. 8-16	4,694 3,049	43.8 13.9	0.17 5.77	9,268 7,263	44.8 10.8	0.21 9.54

actual numbers of individuals of the several kinds are not given; only their percentages of the total, which are essential to quick comparison, are indicated. Any one who wishes to know the numbers can readily calculate them from the total. Though this paper is concerned only with intermediate individuals, the percentages of normal winged aphids is also given for the sake of a comparison to be made on a later page.

From Table I it is clear that more intermediate-winged aphids of this strain were produced in intermittent light than in continuous. This is true for both wingless and winged parents, for every stock, and for all temperatures both before and during the experiments. That is, in every combination of other agents, intermittent light yielded more intermediates than did continuous light. Moreover, the differences are large ones; in no instance is the ratio less than 10:1, and in many sets of conditions the intermediates were 20 or even 50 times as numerous in intermittent as in continuous light. Evidently continuity or discontinuity of light is of major importance in the production of intermediates in the 1923 strain.

Effect of Current Temperature in the 1923 Strain

The results of the experiments with the 1923 strain are rearranged in Table II so as to put the temperatures used during the experiment (24° and 14°) in the fourth column, next to the data, thereby contrasting most sharply the effects of these current temperatures.

The last column of this table, relating to those reared in intermittent light, shows the most striking contrast. In every combination of other conditions, more intermediate-winged offspring were produced at 14° than at 24° . The differences are fairly large, the ratios ranging roughly from 1.3:1 up to nearly 4:1. These differences are obtained, however, only in intermittent light.

In continuous light (column 7), the differences are small and mostly in the opposite direction. That is, more intermediates are produced at 24° than at 14° (one exception). Though all the numbers are small, the fact that nearly all the differences are of the same sign can hardly mean other than that there is a significant reversal of the effect of temperature in continuous light as compared with the temperature effect in intermittent light.

Wingless and Winged Parents in the 1923 Strain

By rearranging the experiments with the two types of parents at the right of the branching table, as is done in Table III, whatever difference there is in the tendency of wingless and winged parents to produce intermediate-winged offspring is best shown.

TABLE II

The data of Table I rearranged to show the contrast between 24° and 14° temperature in the production of intermediate-winged aphids by the 1923 strain.

Nature of parents	Stock parents came from	Temperature of parents preceding experiment	Temperature of parents during experiment	Offspring					
				Produced in continuous light			Produced in 8-hr. light, 16-hr. darkness		
				Total number of off-spring	Percentage winged	Percentage intermediate-winged	Total number of off-spring	Percentage winged	Percentage intermediate-winged
Wingless	24°	24°	24°	7,640	50.7	0.26	5,832	32.5	3.23
			14°	7,835	46.5	0.18	5,592	16.6	5.17
		Room	24°	2,522	47.4	0.12	1,650	38.4	3.03
	14°		14°	2,387	62.7	0.08	2,445	15.4	4.05
		14°	24°	6,550	49.4	0.11	5,240	47.4	5.42
			14°	7,182	55.0	0.07	5,307	20.4	8.95
		Room	24°	880	51.1	0.11	846	39.7	5.52
	Alt.		14°	1,238	50.8	0.00	1,017	18.6	7.37
		Alt.	24°	5,581	49.3	0.13	5,547	39.5	2.94
			14°	7,257	35.8	0.12	5,702	13.0	7.87
Winged	24°	Room	24°	2,902	44.9	0.21	2,709	35.4	3.03
			14°	3,225	44.6	0.09	2,760	17.8	8.48
		Room	24°	4,415	46.8	0.18	3,912	41.1	2.94
	14°		14°	4,694	43.8	0.17	3,049	13.9	5.77
		24°	24°	12,816	59.7	0.37	6,777	37.0	4.01
			14°	17,056	42.4	0.28	17,335	19.2	6.35
		Room	24°	2,261	40.0	0.04	1,737	28.6	4.38
	Alt.		14°	2,301	32.2	0.22	1,647	9.0	10.75
		14°	24°	8,309	33.6	0.13	7,085	20.5	2.87
			14°	6,806	21.3	0.13	4,084	11.5	11.75
		Room	24°	3,329	42.4	0.24	3,326	29.8	2.56
	Room		14°	3,630	29.6	0.08	3,169	10.4	9.09
		Alt.	24°	10,505	55.3	0.39	9,205	43.7	4.20
			14°	12,786	39.6	0.13	8,975	12.9	10.92
		Room	24°	4,094	35.8	0.07	3,545	25.3	2.03
	Room		14°	4,650	34.1	0.04	3,190	7.4	7.62
		Room	24°	8,015	45.8	0.29	6,821	30.3	4.31
			14°	9,268	44.8	0.21	7,263	10.8	9.54

This difference, though sometimes striking, is not everywhere of the same sign. In 21 of the 28 sets of conditions the winged parents produced the more intermediate offspring, and most of the differences are of some size. Of the 7 exceptions to this rule, 6 are from parents which were reared at room temperature throughout their immature stages, suggesting that perhaps the peculiarly variable conditions thus introduced were favorable to intermediate-winged offspring. However, the seventh exception (which is the seventh pair in the left side of Table III) was exhibited by parents not reared at room temperature; and the difference shown here is one of the more striking ones in the table. It seems impossible to bring the wingless and winged aphids under any general rule concerning their tendency to produce intermediate-winged offspring.

Effect of Antecedent Temperatures in 1923 Strain

Two other rearrangements of the results of the experiments would be required to show most directly the effects of the temperatures applied to the aphids prior to the beginning of the experiments. One table would show any difference in the number of intermediate offspring produced by aphids taken from the several temperature stocks, that is, the stocks reared at 24°, 14°, alternating, and room temperatures respectively. The other rearrangement would show whether bringing the parents to room temperature through all their immature stages before using them in an experiment affected the number of their intermediate-winged offspring. The differences which these tables would show are not regular enough to warrant their presentation in that form. A statement of them, however, should be made.

Keeping the experimental parents at room temperature through their immature stages, when that represented a change from previous conditions (as it would for aphids taken from the 24°, 14°, and alternating-temperature stocks), caused them to produce fewer intermediate-winged offspring in 16 out of 24 combinations of other conditions. Of the 8 which showed the opposite effects, 6 were reared at 24° during the experiment, the other 2 were reared at 14°. Among these 8 likewise were aphids from each of the four temperature stocks. Again it is impossible to state a general rule describing the effect of a one-generation transfer to room temperature in advance of an experiment.

Still less regular is the difference between the various temperature stocks, in their tendency to produce intermediate-winged offspring. Aphids from each stock yielded more intermediates than did those from all the other stocks, in from one to three of the combinations of other conditions. This is true regardless of whether the aphids were brought

to room temperature for one generation before the experiment or not. Aphids changed from the alternating-temperature stock to room temperature yielded more than did those reared continuously at room tem-

TABLE III

Differences between wingless and winged parents of the 1923 strain in their production of intermediate-winged offspring under various conditions. (*ws* = wingless, *wd* = winged.)

Temperature of parents preceding experiment	Light on parents during experiments	Temperature of parents during experiments	Stock parents came from	Nature of parents	Total number of offspring	Percentage of offspring winged	Percentage of offspring intermediate-winged	Temperature of parents preceding experiment	Light on parents during experiments	Temperature of parents during experiments	Stock parents came from	Nature of parents	Total number of offspring	Percentage of offspring winged	Percentage of offspring intermediate-winged	
24°	Cont.	24°	24°	<i>ws</i>	7,640	50.7	0.26	Room	Cont.	24°	24°	<i>ws</i>	2,522	47.4	0.12	
			<i>wd</i>	12,816	59.7	0.37					<i>wd</i>	2,261	40.0	0.04		
		14°	24°	<i>ws</i>	7,835	46.5	0.18				14°	<i>ws</i>	880	51.1	0.11	
				<i>wd</i>	17,056	42.4	0.28					<i>wd</i>	3,329	42.4	0.24	
	8-16	24°	24°	<i>ws</i>	5,832	32.5	3.23				Alt.	<i>ws</i>	2,902	44.9	0.21	
				<i>wd</i>	6,777	37.0	4.01					<i>wd</i>	4,094	35.8	0.07	
14°	Cont.	14°	24°	<i>ws</i>	5,592	16.6	5.17				14°	Room	<i>ws</i>	4,415	46.8	0.18
				<i>wd</i>	17,335	19.2	6.35							<i>wd</i>	8,015	45.8
		24°	14°	<i>ws</i>	6,550	49.4	0.11			24°			<i>ws</i>	2,387	62.7	0.08
				<i>wd</i>	8,309	33.6	0.13						<i>wd</i>	2,301	32.2	0.22
	8-16	14°	14°	<i>ws</i>	7,182	55.0	0.07			14°		<i>ws</i>	1,238	50.8	0.00	
				<i>wd</i>	6,806	21.3	0.13					<i>wd</i>	3,630	29.6	0.08	
Alt.	Cont.	24°	14°	<i>ws</i>	5,240	47.4	5.42		8-16	24°		Room	<i>ws</i>	4,694	43.8	0.17
				<i>wd</i>	7,085	20.5	2.87						<i>wd</i>	9,268	44.8	0.21
	8-16	14°	14°	<i>ws</i>	5,307	20.4	8.95				24°	<i>ws</i>	1,650	38.4	3.03	
				<i>wd</i>	4,084	11.5	11.75					<i>wd</i>	1,737	28.6	4.38	
	Cont.	24°	Alt.	<i>ws</i>	5,581	49.3	0.13			14°	Room	14°	<i>ws</i>	846	39.7	5.52
				<i>wd</i>	10,505	55.3	0.39						<i>wd</i>	3,326	29.8	2.56
8-16	14°	Alt.	<i>ws</i>	7,257	35.8	0.12	Alt.					<i>ws</i>	2,709	35.4	3.03	
			<i>wd</i>	12,786	39.6	0.13						<i>wd</i>	3,545	25.3	2.03	
Alt.	Cont.	24°	Alt.	<i>ws</i>	5,547	39.5	2.94		14°		Room	Room	<i>ws</i>	3,912	41.1	2.94
				<i>wd</i>	9,205	43.7	4.20						<i>wd</i>	6,821	30.3	4.31
	8-16	14°	Alt.	<i>ws</i>	5,702	13.0	7.87					24°	<i>ws</i>	2,445	15.4	4.05
				<i>wd</i>	8,975	12.9	10.92						<i>wd</i>	1,647	9.0	10.75
	Cont.	24°	Alt.	<i>ws</i>	7,257	35.8	0.12			14°	Room	14°	<i>ws</i>	1,017	18.6	7.37
				<i>wd</i>	12,786	39.6	0.13						<i>wd</i>	3,169	10.4	9.09
8-16	24°	Alt.	<i>ws</i>	5,547	39.5	2.94	Alt.					<i>ws</i>	2,760	17.8	8.48	
			<i>wd</i>	9,205	43.7	4.20						<i>wd</i>	3,190	7.4	7.62	
Cont.	24°	Alt.	<i>ws</i>	5,702	13.0	7.87	14°		Room		Room	<i>ws</i>	3,049	13.9	5.77	
			<i>wd</i>	8,975	12.9	10.92						<i>wd</i>	7,263	10.8	9.54	

perature in some combinations of conditions, but fewer in other combinations.

Antecedent temperatures on the whole thus give mixed results in the

production of intermediate-winged offspring. All these relations can be made out from the tables already given, though the numbers contrasted are not in adjoining lines. It seems not worth while to bring them thus together in view of the impossibility of generalizing from them.

Wing Production and Intermediate Wings in the 1923 Strain

It is worthy of note that in general the greatest production of intermediate wings does not accompany the greatest production of wings. Intermittent light, with every combination of the other factors, resulted in more intermediate-winged but fewer winged individuals, than did continuous light (Table I). Current temperature of 14° , with intermittent light, produced likewise more intermediate-winged but fewer winged offspring than did 24° , in every combination of other factors (Table II, right). However, current temperature of 14° , with continuous light, produced mostly fewer intermediate-winged and at the same time mostly fewer winged offspring than did 24° (one or two exceptions, Table II, left half). The differences here are mostly small, however, for both the winged and the intermediate-winged.

Within any one of these major groups, the relation between wing production and intermediate wings may also be determined from their correlation. This has been calculated for the two halves of Table II separately. For the right half of that table, where production of intermediate wings is everywhere high and that of normal wings moderate, the correlation between the two is found to be $r = -0.68 \pm 0.10$. This means that in general the fewer the wings the more numerous the intermediate wings. This negative relation is not due, however, to a mere conversion of wings into half-grown wings at low temperature. Even if all the intermediate-winged aphids were converted into winged ones, there would still be in most conditions a considerable deficit of winged individuals at the lower temperature.

For the left half of Table II the corresponding correlation between wings and intermediate wings is $r = 0.26 \pm 0.18$, which is positive but not of proven significance. There is no simple relation, therefore, between production of wings and production of intermediates; but on the whole these two things are oppositely affected by the same sets of conditions.

The bearing of these facts on the nature of intermediates is discussed in a later section.

The 1931 Strain of Aphids

The 1931 strain of aphids differs very considerably from the 1923 strain in the production of both winged and intermediate-winged individuals. The proportion of intermediate aphids in the 1931 strain is only about one-eleventh of that in the 1923 line. In wing production, the 1931 strain resembles the 1923 strain during the latter's first six years; that is, before its "mutation" of 1929. The 1931 line is thus more like clone A than like clone A' of a former paper (Shull, 1932). Both clone A and the 1931 strain produced more winged individuals in intermittent than in continuous light; and when the relatively weak light used in the recent experiments is taken into account, the degree of their response to light is probably not very different.

Effect of Continuous and Intermittent Light on Intermediates in the 1931 Strain

The experiments with the 1931 strain are first presented with the contrast between continuous and intermittent light most plainly shown, as in Table IV. In 27 of the 28 pairs of percentages of intermediate-winged offspring the larger percentage is that obtained in intermittent light. The numbers are mostly small, but the fact that in all but one pair the differences are in the same direction indicates the significance of the result.

The only situation in which the difference is very marked is that involving a succession of 24°, room, and 14° temperatures; that is, in which aphids from the 24° stock were put at room temperature where their offspring grew to maturity, and these offspring became the parents in the experiment at 14°. Both wingless and winged parents responded in the same decisive way, hence the large number of intermediate offspring can hardly be accidental.

The one exception to the direction of difference arose from a situation like that above except that aphids from the 14° stock were used. The preceding temperature (room) and the current temperature (14°) were the same. The difference was small, and the reversal of its sign is found only among the offspring of winged parents; hence it is of questionable meaning.

Effect of Antecedent Temperatures on Intermediates in the 1931 Clone

By rearranging the results obtained with the 1931 strain as in Table V, the effect of temperatures applied to the ancestors of the ex-

TABLE IV

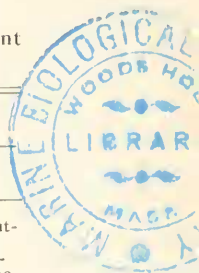
The percentage of winged and intermediate-winged offspring produced by both wingless and winged aphids of the 1931 strain in different combinations of light and temperature both during and before the experiments.

Temperature of parents during experiment	Stock parents came from	Temperature of parents preceding experiment	Light on parents during experiment	Offspring					
				From wingless parents			From winged parents		
				Total number of offspring	Percentage winged	Percentage intermediate-winged	Total number of offspring	Percentage winged	Percentage intermediate-winged
24°	24°	24°	Cont. 8-16	6238 5827	11.5 26.8	0.06 0.46	6332 5055	6.7 18.8	0.13 0.69
		Room	Cont. 8-16	2094 2063	32.8 29.3	0.00 0.58	1777 1659	20.8 25.8	0.06 0.60
	14°	14°	Cont. 8-16	6742 6169	4.8 33.9	0.06 0.24	3411 3315	1.0 20.2	0.09 0.72
		Room	Cont. 8-16	2758 2913	11.5 32.4	0.11 0.27	1824 1707	4.6 17.3	0.00 0.06
	Alt.	Alt.	Cont. 8-16	3720 3642	7.2 10.2	0.05 0.14	5304 5144	7.9 22.5	0.23 0.45
		Room	Cont. 8-16	3945 2546	12.4 26.8	0.03 0.31	1412 980	3.0 7.4	0.00 0.20
	Room	Room	Cont. 8-16	6709 5231	21.0 33.5	0.18 0.98	6480 5214	7.0 18.4	0.23 0.98
14°	24°	24°	Cont. 8-16	6306 5187	14.7 75.7	0.08 0.42	6587 5506	10.7 75.8	0.08 0.64
		Room	Cont. 8-16	2593 2196	43.0 53.6	0.12 2.46	2086 1557	39.8 37.9	0.10 4.82
	14°	14°	Cont. 8-16	7356 6074	9.4 82.6	0.05 0.36	3990 3381	3.0 67.7	0.00 0.62
		Room	Cont. 8-16	2874 2473	12.6 85.5	0.14 0.36	1586 1712	9.6 84.0	0.06 0.00
	Alt.	Alt.	Cont. 8-16	4358 3774	13.6 89.1	0.02 0.19	5735 5088	3.1 83.7	0.04 0.35
		Room	Cont. 8-16	4024 3134	16.4 86.8	0.05 0.10	1441 1179	3.3 84.8	0.00 0.08
	Room	Room	Cont. 8-16	6463 6041	14.6 80.6	0.11 0.53	6308 5012	10.1 75.1	0.32 1.06

TABLE V

The data of Table IV rearranged to show the effect of different antecedent temperatures applied to the parent aphids of the 1931 clone.

Light on parents during experiment	Temperature of parents during experiment	Stock parents came from	Temperature of parents preceding experiment	Offspring					
				From wingless parents			From winged parents		
				Total number of offspring	Percentage winged	Percentage intermediate-winged	Total number of offspring	Percentage winged	Percentage intermediate-winged
Cont.	24°	24°	24°	6238	11.5	0.06	6332	6.7	0.13
			Room	2094	32.8	0.00	1777	20.8	0.16
		14°	14°	6742	4.8	0.06	3411	1.0	0.09
			Room	2748	11.5	0.11	1824	4.6	0.00
	Alt.	Alt.	Alt.	3720	7.2	0.05	5304	7.9	0.23
			Room	3945	12.4	0.03	1412	3.0	0.00
		Room	Room	6709	21.0	0.18	6480	7.0	0.23
	14°	24°	24°	6306	14.7	0.08	6587	10.7	0.08
			Room	2593	43.0	0.12	2086	39.8	0.10
		14°	14°	7356	9.4	0.05	3990	3.0	0.00
			Room	2874	12.6	0.14	1586	9.6	0.06
8-16	24°	Alt.	Alt.	4358	13.6	0.02	5735	3.1	0.04
			Room	4024	16.4	0.05	1441	3.3	0.00
		Room	Room	6463	14.6	0.11	6308	10.1	0.32
	Alt.	24°	24°	5827	26.8	0.46	5055	18.8	0.69
			Room	2063	29.3	0.58	1659	25.8	0.60
		14°	14°	6169	33.9	0.24	3315	20.2	0.72
			Room	2913	32.4	0.27	1707	17.3	0.06
	Room	Alt.	Alt.	3642	10.2	0.14	5144	22.5	0.45
			Room	2546	26.8	0.31	980	7.4	0.20
		Room	Room	5231	33.5	0.98	5214	18.4	0.98
	14°	24°	24°	5187	75.7	0.42	5506	75.8	0.64
			Room	2196	53.6	2.46	1557	37.9	4.82
		14°	14°	6074	82.6	0.36	3381	67.7	0.62
			Room	2473	85.5	0.36	1712	84.0	0.00
	Alt.	Alt.	Alt.	3774	89.1	0.19	5088	83.7	0.35
			Room	3134	86.8	0.10	1179	84.8	0.08
	Room	Room	Room	6041	80.6	0.53	5012	75.1	1.06



perimental aphids is shown. The last column preceding the actual data shows the temperature applied to just one generation prior to the beginning of the experiments. Since, however, there is some coupling of the temperature applied to that generation with the temperature of preceding generations (not all combinations having been tried), it is a combination of the last two (third and fourth) columns of the branching part of the table to which the data are most closely related.

This arrangement shows that aphids taken from the room temperature stock yielded more intermediate-winged offspring than did those of any of the stocks kept at other temperatures (24° , 14° , alternating), provided the stock conditions were maintained up to the very beginning of the experiment. This is true of both wingless and winged parents, and of every combination of other conditions, though one of the differences is so slight as not to appear as a difference in the table.

Changing the parents to room temperature from one of the other temperatures (24° , 14° , or alternating) yielded on the whole more intermediate-winged offspring than did continuing the stock temperature up to the beginning of the experiments, if wingless parents were used, but fewer intermediates if winged parents were used. There are, however, three exceptions out of twelve to each part of that statement. Moreover, one of the exceptions is one of the only two very striking differences to which attention was called in the preceding section. These exceptions, which do not themselves possess any one characteristic in common, weaken the generalization that seems otherwise warranted.

*Effect of Temperature and of Nature of Parents on Production
of Intermediates in 1931 Strain*

The remaining factors produce too little or too variable an effect on intermediacy to warrant rearrangement of the data in tables to show those effects clearly. The following statements of these irregular effects can be verified from the tables already given, by comparing percentages not adjacent to one another.

The nature of the parents has no regular influence on the number of intermediates. Winged parents produced more intermediate offspring in 17 combinations of external conditions, but fewer in 11 combinations. Eight of the latter group of 11 came from 14° and alternating temperature stocks and in addition were at room temperature for one generation before the experiment started.

Current temperature had no consistent effect, since aphids at 14° produced more intermediates than did those at 24° in 13 combinations, but fewer in 14, and an equal number in 1. No single factor is common to all of the 13, nor to all of the 14.

*Relation of Intermediacy to Wing Production in the
1931 Strain*

As was stated earlier, the only situation in which the 1931 clone produced very many intermediate-winged individuals was intermittent light and a current temperature of 14°, applied to parents which came from the 24° stock but which were reared at room temperature throughout their own immature stages (eighteenth line, Table IV).

These conditions are not exceptionally conducive to production of normal-winged aphids. Winged individuals were, it is true, more abundant (47 per cent) under these conditions than in the experiments as a whole (34 per cent) in the 1931 strain. But other combinations of conditions resembling this one yielded higher wing production as often as lower. Thus, changing only the current light from intermittent to continuous served to reduce wing production 13 per cent; and changing only the current temperature from 14° to 24° reduced wing production 19 per cent. But omitting only the change to room temperature during the immature stages of the parents in the experiments increased wing production 29 per cent; and taking the parents from the 14° or the alternating temperature stock instead of the 24° stock increased wing production 38 per cent and 39 per cent respectively. None of these percentages appears directly in any of the tables, since they are based on the combined output of both wingless and winged parents.

It may also be pointed out that the set of conditions yielding the most intermediate individuals produced fewer winged ones than any other set of conditions that included a current temperature of 14°, but more than any set involving 24° temperature during the experiments.

On the whole, therefore, the greatest number of intermediate aphids would seem to be produced under circumstances which favored medium wing production in this strain.

Less connection between wings and intermediate wings is to be seen in a comparison of lines 17 and 18 in Table IV. The two very large percentages of intermediates are shown in line 18. The conditions resulting in the aphids of line 17 differ from those producing the aphids of line 18 only in that they included continuous light instead of intermittent light. This one change reduced the proportion of winged aphids only moderately, but reduced the intermediates very greatly. Continuous light reduces the number of winged aphids in some other clones (clone A, for example, Shull, 1932), and in general does so for the 1931 strain used in these experiments. That response is well shown in Table V, where the percentages of winged individuals are consistently lower in the upper half of the table than in the lower. But in that

upper half, the percentages in the ninth line stand out as far higher than any others. Continuous light had little of its usual effect on the number of winged offspring in this particular combination of other agents, but reduced the intermediate-winged individuals to as low a proportion as the average of all others in continuous light (upper half of Table V). It is possible, therefore, to change the production of intermediates without greatly changing the production of normal-winged aphids.

If the relation of intermediates to winged aphids is to be studied by the correlation method, the data of the 1931 strain must be broken up into three parts. Since intermittent light favors both wings and intermediate wings, strong correlation between the two would be shown by the data as a whole, but this would indicate nothing more than Table IV does. Current temperature also has a marked effect on wing production in intermittent light, though not in continuous light. Since calculation of correlation coefficients is justified only when the two variable qualities are distributed unimodally, and is most significant when the distribution is fairly symmetrical, separation of the data into three groups is necessary to avoid wide gaps and extreme skewness. The three groups are (1) those aphids reared in intermittent light and at 24°, (2) those reared in intermittent light at 14°, and (3) those reared in continuous light without distinction of temperature. From two of these have been omitted two percentages (winged aphids in one, intermediates in the other) because they were so high as to create the discontinuity which grouping the data was designed to prevent. These high percentages must have some special reasons with which correlation studies are not fitted to deal. The three coefficients of correlation between percentage of winged and percentage of intermediate-winged are, respectively, $.30 \pm .24$, $-.70 \pm .15$, and $.03 \pm .20$. Two are insignificant, the third strongly negative.

It seems clear, therefore, that whatever is inducing wing development in these more unified segments of the experiments is not particularly conducive to production of intermediates—is, indeed, somewhat opposed to it.

Conceivable Schemes of Stimulation

The general method of explaining intermediates proposed by Goldschmidt (1923 et al.) particularly for intersexes is perhaps applicable to aphids. It involves the assumption that something (a hormone) responsible for the development of structural characters is present in different concentrations at different times, usually in increasing concentrations with increasing age during the critical period of development.

Since the sexes start development under the influence of different groups of genes, and since they typically possess in the end quite different structures, it was natural to assume two hormones, one for each sex. Development of the individual began with one of these hormones in the ascendancy, but somewhere in the course of differentiation it was overtaken by the other hormone, the moment of passing being the "turning point." Structures determined before that point were like those of one sex, characters determined after that point were like those of the other sex.

It would be possible to make the same assumptions regarding intermediates between winged and wingless aphids. These aphids, however, are presumably genetically alike. Moreover, the difference between them lies almost entirely in the possession by the winged type of a group of characters which are wanting in the wingless. It is therefore simpler to represent the differentiating characters as dependent upon the concentration of a single substance in the critical period of development. No serious flaw would be created in the concept of the fundamental nature of the intermediates even if this representation should prove to be in error.

It is assumed for the purpose of this discussion that this one hormone varies in concentration during development in the general manner portrayed by Goldschmidt's curves, which were originally suggested, no doubt, by the course of other biological processes in developing organisms. In proper concentrations it will be regarded as a stimulus to the development of the differential characters of the winged type, though it could as well be considered an inhibitor. The unsegmented egg will be represented as possessing only a minimal quantity of this hormone, though it is conceivable that the egg might have a higher concentration than any later stage. Within these self-imposed limits we may imagine the influence which various situations would have on the frequency and nature of intermediates.

Differentiating Characters.—The winged female aphid differs from the wingless in having wings, three ocelli on the head, wing muscles in the thorax, and extra sensoria on the third segment of the antennæ (4–6 in the wingless, 15–18 in the winged). There are color differences, the third segment of the antennæ being blackish and the thorax brownish in the winged type, but these are subject to considerable variation and are not included in this discussion. It is the four structural characters named which are assumed to develop if a certain concentration of the stimulating hormone is attained early enough.

Sharply Defined Single Thresholds.—The line of demarcation between wingless and winged could be a sharply defined level of concentra-

tion of the hormone, such that any deficit, however slight, would leave the differentiating structures wholly absent, while any excess, however small, over this level would cause those structures to be fully developed. The simplest case would be that in which the same concentration of the hormone would stimulate the development of all four structures—wings, ocelli, wing muscles, and sensoria.

The time in development at which these structures have their fate (their presence or absence) decided could likewise be a sharply defined moment, such that if the stimulus were applied in advance of that time the structures would develop, while if the stimulating level of the hormone were attained even only slightly after that time the organs would be entirely lacking in the adult. The simplest case is again that in which the time of determination is the same moment for all four of the structures.

This situation is represented in Fig. 1, *A*, in which time during development is expressed by horizontal distances and concentration of the hormone by vertical distances. The curves indicate the rate of accumulation of the hormone during development. Any curve passing to the left of and above the dot in the middle of the chart would be that of a winged adult; any curve below and to the right, that of a wingless adult.

Intermediate individuals would be practically excluded by the relations here described, since very seldom would a curve of concentration pass exactly through the intersection of stimulation level and time of determination.

Different Times of Determination.—The preceding situation could be modified by having different times of embryonic determination for the four structures, but the same concentration of the hormone as a stimulus for all. This condition is represented in Fig. 1, *B*. The order of determination of the four differentiating features is arbitrarily assumed purely for purposes of illustration. All curves of hormone concentration rising rapidly enough to pass the stimulating level in advance (to the left) of all these times of determination would be those of winged aphids; while all curves reaching that level to the right of (after) all the times of determination would be those of wingless individuals. Curves between these limits would lead to mosaics. The second curve in *B* would be that of an aphid having ocelli, wing muscles and extra antennal sensoria fully developed, but lacking wings entirely. The third curve would pertain to one lacking wings and ocelli altogether, but having wing muscles and extra sensoria fully present. The fourth curve would belong to an aphid having extra antennal sensoria to the full number, but lacking all the other marks of a winged individual.

It is proposed in this paper to use the term mosaic for a patchwork of typical parts, the word intermediate for an individual in which one

or more structures are partially developed. "Typical parts" means, in wingless aphids, the absence of any degree of the marks of a winged aphid. A mosaic thus would have one or more of the features of the winged aphid completely developed, but lack the others entirely. One individual could be both mosaic and intermediate, by having certain structures either fully developed or wanting, other structures partially developed.

The conditions described in *B* would lead to a certain number of mosaics, but rarely to intermediates. How many mosaics there were would depend partly on the lengths of the intervals between the times of determination.

Different Levels of Stimulation.—The several features of a winged aphid could be stimulated to develop by different concentrations of the hormone. If there were a single time of determination for all four of these features, the general situation would be as shown in Fig. 1, *C*. Without further explanation it should be clear that the second and third curves are those of mosaics, the second possessing ocelli, wing muscles and extra sensoria, the third having only wing muscles and sensoria. Intermediates would be rare, and the number of mosaics would depend on the extent of the differences among the levels of stimulation.

Range of Stimulation.—It seems likely that a certain concentration of the hormone could not act suddenly to determine a complete structure. More probably a given concentration, if applied early enough, serves to stimulate a slight development of the structure, while a greater concentration would cause a greater development of it. There would thus be a level (concentration) of beginning differentiation, and a level of complete differentiation. If this range of stimulation were identical for all four of the features of the winged aphid, and if there were a sharp time of determination likewise common to all four of them, the conditions would be represented by Fig. 1, *D*. Under these conditions there could be some intermediates, but no mosaics. Any individual whose hormone concentration rose above the level of beginning differentiation before the time of determination, but did not rise as high as the level of complete differentiation until after that time, would be an intermediate. All four of the distinguishing structures would be only partially developed. They might not be developed to equal degrees, since they might respond at different rates to increasing quantities of the hormone. The second curve in *D* would belong to an aphid having wings, ocelli, wing muscles, and antennal sensoria highly, but not completely, developed, the third curve to one in which these structures were only slightly differentiated.

How many intermediates were produced would depend in part on

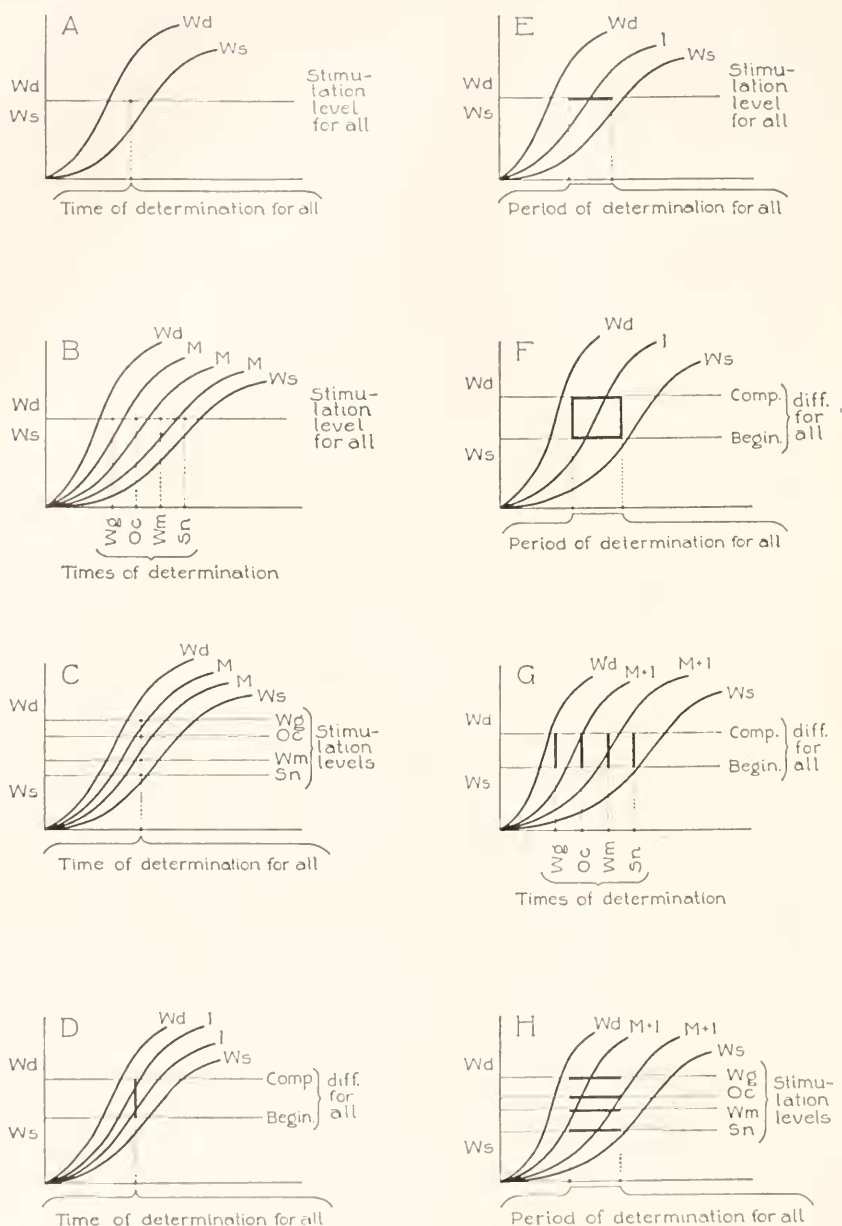


FIG. 1. Curves representing hormone concentration in relation to production of intermediate-winged individuals in aphids, under various assumptions concerning times or periods of determination and levels or ranges of stimulation during embryonic development. I , intermediate; M , mosaic; O_c , ocelli; S_n , sensoria; W_d , winged; W_g , wings; W_m , wing muscles; W_s , wingless.

the difference between the concentration which would begin and that which would complete the differentiation.

Period of Determination.—It is not necessary that a structure have its fate irrevocably fixed at one moment in development. That determination may be spread over a period of time. If the hormone rises to a stimulating concentration just before that period ends, the structure may be supposed to be slightly developed. If the hormone reaches a stimulating level soon after the beginning of the period of determination, the structure would be highly but not completely developed. In Fig. 1, *E*, it is assumed that there is one period of determination for all structures and one sharp level of stimulation for all of them.

The above situation would lead to the production of some intermediates, but no mosaics. The middle curve of *E* would be that of an aphid having perhaps half-developed wings, ocelli, wing muscles, and extra sensoria. How many intermediates there were would depend on the length of the period of determination.

Range of Stimulation and Period of Determination.—There may conceivably be a range of stimulation levels (as in *D*) and also a period of determination (as in *E*). The simplest such situation is one in which all four differentiating structures would be stimulated by the same range of concentrations of the hormone and be determined during the same period of time. It is represented in Fig. 1, *F*. Any curve of hormone concentration which passed through the rectangle formed by the intersection of the range of stimulation and the period of determination would be that of an intermediate. All the differentiating structures of such an individual would be partially developed, though not necessarily to an equal extent. There would be no mosaics. How many intermediates were produced would depend partly on the size of the rectangle—the extent of the range of stimulation and the length of the period of determination.

Range of Stimulation with Different Times of Determination.—If there be one range of stimulating concentrations of the hormone common to all four of the structures, and sharply defined times of determination which are different for all four, the conditions are those portrayed in Fig. 1, *G*. Any curve cutting one of the heavy vertical lines would belong to an individual intermediate for the structure whose time of determination and range of stimulation that heavy line represents. The same individual would be mosaic for any structures whose heavy line its curve did not cut. Thus, the second curve in *G* would be that of an aphid lacking wings, having almost complete ocelli, and having fully developed wing muscles and sensoria. The third curve would belong to an individual lacking wings and ocelli, but having partially developed wing muscles and fully developed sensoria.

Individuals not typically winged or wingless, produced under the above conditions, might well be both mosaic and intermediate. Some of them would perhaps be mosaic alone, especially if the range of stimulation were narrow and the times of determination were widely spaced. Most of them would be intermediate only, if the range of stimulation were broad and the intervals between times of determination were very short.

Common Period of Determination with Different Levels of Stimulation.—Another possibility is that all structures have the same period of determination but different sharply defined levels of stimulation, as in Fig. 1, *H*. An individual would be intermediate for a given character if its hormone curve cuts the heavy line representing its period of determination and level of stimulation. It would be mosaic for (either possess or lack) any structure whose corresponding heavy line it did not cut.

Intermediates would prevail if the period of determination were long and the stimulation levels close together. Mosaics would be more common if the period of determination were short and the levels of stimulation far apart. Many atypical individuals would doubtless be both mosaic and intermediate.

One Level of Stimulation with Distinct Periods of Determination.—A single sharp level of stimulation for all distinguishing structures may be combined with periods of determination which are different for the different characters. If these periods do not overlap, the situation is as pictured in Fig. 2, *I*. The second curve in *I* is that of an individual with partially developed wings and the other three features of a winged aphid fully formed. The third curve is that of a mosaic having wing muscles and sensoria fully developed but wholly lacking wings and ocelli. Intermediates would prevail among atypical individuals if periods of determination were long and close together; mosaics would lead in numbers if periods of determination were short and separated by wide intervals.

Single Time of Determination with Separate Ranges of Stimulation.—If all the differentiating structures were determined at the same moment, but each had its own range of stimulation which did not overlap any of the others, the situation would be that represented by Fig. 2, *J*. The second curve of this chart, cutting one of the heavy lines representing time of determination and range of stimulation but missing the others, represents a mosaic and intermediate individual. The third curve, missing all the heavy lines, is that of a mosaic only. Intermediates would be favored, among atypical aphids, by wide ranges of stimulation close together; mosaics would be favored by narrow ranges widely spaced.

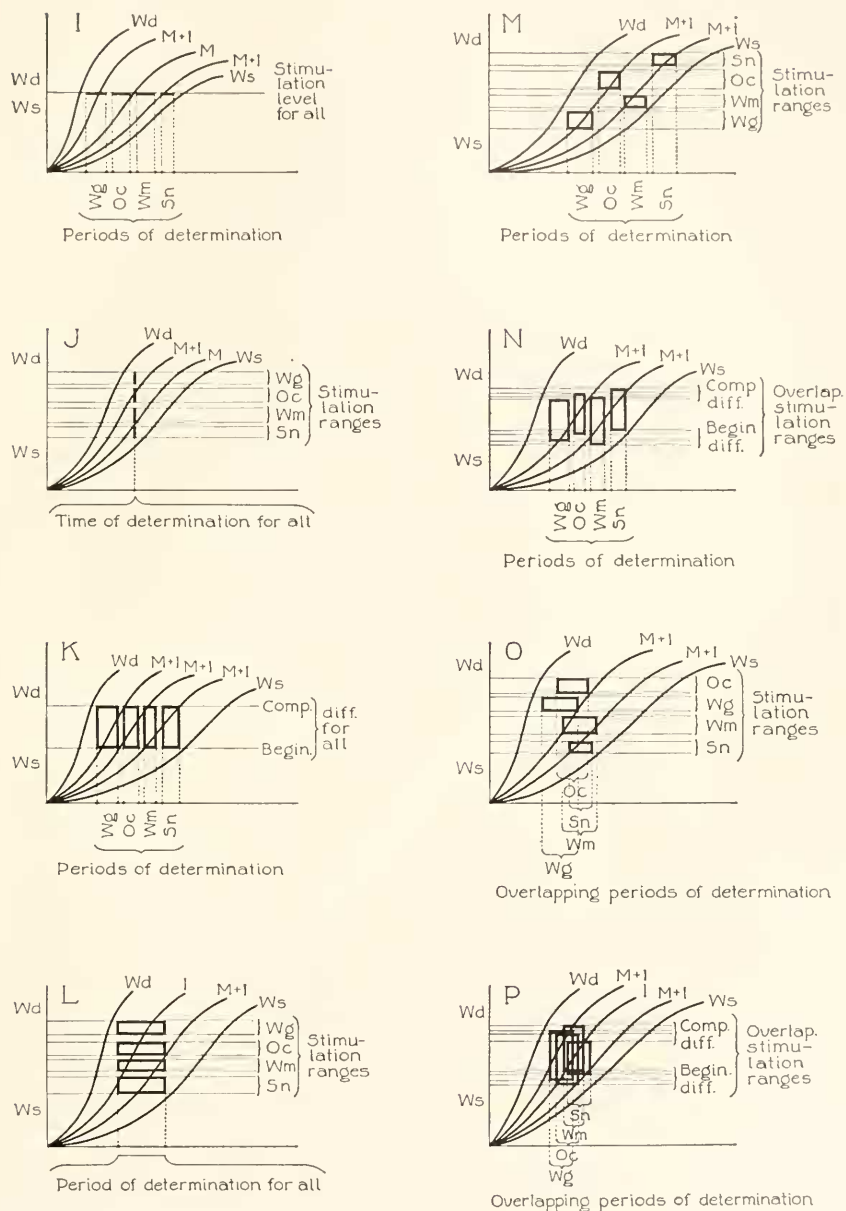


FIG. 2. Continuation of Fig. 1, representing increasing substitution of ranges for levels of stimulation, and of periods for times of determination, and increasing overlapping in place of distinct ranges and periods.

One Range of Stimulation with Distinct Periods of Determination.—If stimulation occupied a range common to all structures, and each organ had a different period of determination without overlapping, Fig. 2, *K*, would represent the conditions. The three curves in the middle are those of both mosaics and intermediates, since each curve cuts one or more of the heavy rectangles and misses one or more others. If the rectangles were narrow and high and close together laterally (that is, if periods of determination were short and not far apart while the threshold of complete differentiation were far above the threshold of beginning differentiation), most atypical individuals would be intermediate only. If periods of determination were short and widely separated, and the range of stimulation were narrow, mosaics would predominate.

Common Period of Determination with Distinct Ranges of Stimulation.—The situation indicated by this title is portrayed in Fig. 2, *L*. Individuals would be intermediate for those characters whose rectangles their hormone curves traverse, mosaic for those whose rectangles are missed altogether. If the common period of determination were long, and the ranges of stimulation were narrow and close together, most atypical aphids might well be intermediate only. In most other situations of this general kind, combined mosaics and intermediates would be more likely.

Distinct Periods of Determination and Separate Ranges of Stimulation.—In Fig. 2, *M*, is shown the combination of different periods of determination and different ranges of stimulation, without any overlapping of either periods or ranges. Many possibilities for either few or many intermediates, few or many mosaics, are presented by this situation. If the structures having early determination have low stimulation ranges, and those determined late have high stimulation ranges, so that the rectangles are arranged along a line rising obliquely to the right (as is more or less true in *M*), and if the curve of concentration of the hormone follows approximately this same course, the curves would cut most of the determination-stimulation rectangles if they cut any of them, and mostly intermediates would result. An atypical individual might easily be intermediate for all the distinguishing characters. If, however, structures determined early had high ranges of stimulation, and those determined late had low ranges of stimulation, so that in the figure the rectangles of determination and stimulation were arranged along a line sloping downward to the right, cutting the curve of hormone concentration nearly at a right angle, mosaics would prevail if the rectangles were small, and mosaic-intermediates would be most common if the rectangles were large. Many other relations between the period of determination and range of stimu-

lation could exist, such that the rectangles would be distributed irregularly in relation to the hormone curve. Each of these sets of relations would result in a particular frequency of mosaics or intermediates or both.

Distinct Periods of Determination with Overlapping Ranges of Stimulation.—The opportunity for pure mosaics would be reduced by having the ranges of stimulation overlap. This condition along with distinct periods of determination, is shown in Fig. 2, *N*. Unless the overlapping of ranges were very slight, it would be difficult for the hormone curve to pass between rectangles. While curves near the winged or the wingless extreme might miss some of the rectangles, they could scarcely miss all of them and still belong to atypical individuals.

Overlapping Periods of Determination with Distinct Ranges of Stimulation.—Equally difficult, with the preceding situation, for the production of pure mosaics would be the condition in which the periods of determination overlap while the ranges of stimulation are separate. In Fig. 2, *O*, where this situation is represented, there might appear to be a greater chance for one of the atypical curves to miss all the rectangles than in *N*, but that is only because the periods of determination have been assigned a smaller spread than was given to the ranges of stimulation in *N*. Most atypical aphids produced under these conditions would have to be intermediate with respect to some characters, and could easily be intermediate for all their distinguishing marks.

Overlapping of Both Periods of Determination and Ranges of Stimulation.—Still more favorable to intermediates, as compared to mosaics, than any of the preceding arrangements is that in which both the periods of determination and the ranges of stimulation overlap, as in Fig. 2, *P*. If the limits of the several periods and the several ranges did not differ very greatly, there would be few atypical curves that did not traverse several of the determination-stimulation rectangles, and many would traverse all of the rectangles. Intermediates would thus predominate over mosaics, though individuals both intermediate and mosaic could be fairly common.

The above are not all of the conceivable combinations of stimulation and determination. They will suffice, however, to illustrate the possibilities farther than there is any present possibility of forming judgments from them.

Nature of the Production of Atypical Forms

Since the factors which govern morphogenesis are as yet only very imperfectly understood, the possibilities outlined in the preceding sec-

tion may all be taken into account. What information, if any, concerning them is furnished by the methods of producing the intermediates?

Analysis of the characters of the atypical aphids has only begun, and it is too early to attempt to draw from them the conclusions to which complete study may lead. Already, however, it is clear that most if not all of them are intermediate in one or more respects. To what extent they may also be mosaic is not at present known, but very few of them are pure mosaics.

This fact should mean that the first few situations represented in the curves of Fig. 1 are less likely to exist than are the later ones or those of Fig. 2. Since sharply defined times of determination and levels of hormone stimulation would lead to mosaics, the facts so far ascertained favor the existence of a spread in one or both of these elements. Either there must be a difference between the concentration of the stimulating hormone which will lead to slight development of a structure and the concentration which will complete it, or the amount of development must depend in part on how long the effective stimulation is applied, or both of these statements must be true. Only a more complete knowledge of individual intermediates will indicate how much spread is required.

Until some physiological basis of embryonic determination² in the aphids is discovered, either a range of stimulating concentrations or a gradual process of determination may be postulated to explain intermediate individuals. Which is the more likely, or are both probable?

One fact reported in this paper which appears to bear on this question is the large difference in the capacity of the two strains used to produce intermediates. The 1931 line produced less than one-tenth of the number of intermediates produced by the 1923 strain. Also, this capacity to produce intermediates must be subject to large modifications, since the high capacity of the 1923 line (clone A', Shull, 1932) arose suddenly by a "mutation" from a stock (clone A) which was producing apparently no more atypical individuals than the 1931 strain now produces. Which of the possible bases of intermediacy is more likely to exhibit large differences in different strains, or more likely to be subject to considerable change?

² The word "determination" is used in this paper despite its obvious defects. Since different events occurring at different times may be necessary antecedents of a morphogenetic change, a structure could not be said to be actually determined until the last such event had taken place. A morphological feature may be determined, as far as one agent is concerned, later than it is determined in relation to another agent. Were it possible, in the aphid experiments, to designate a single agent in relation to which determination was being considered, a better term than would not be too cumbersome could be devised. But the tables presented in this paper indicate that a complex set of circumstances is involved. Any substitute for the word determination that would be an improvement upon it would be unwieldy. Biologists are not likely to be misled, in this connection, by the briefer term.

Intermediacy could be made more frequent by increasing the length of the period or periods of determination, or by increasing the range of stimulation, so that more curves of concentration of the hormone would pass through them. It could also be made more abundant by bunching the curves of concentration; that is, by creating a set of conditions which would cause larger numbers of individuals to develop their stimulating hormone at approximately the same rate, which would have to be a rate that would make the curves of concentration traverse the lines or rectangles representing periods of determination or ranges of stimulation or both. This concentration of the hormone curves in a narrow band would have to occur in one strain of aphids, but not in another subjected to the same combination of light and temperature, or any other combination yet tested.

The second of these postulates, the clustering of the rates of hormone development, involves an increase in the measure of control exerted by the animals over the process of development. A strain of aphids in which more individuals developed in the same way would be exercising more regulation than would a line in which hormone increase was more nearly random. Now, the 1923 strain, which is the one in which such clustering of rates of hormone development would have to occur, does not regulate wing production nearly as precisely as does the 1931 line. Wing development responds to light conditions much more definitely in the latter than in the former, as a glance at the respective tables shows. The fact that the effect of intermittent light is reversed in the two strains is not at issue; the significant point is that the effect is much more precise in the 1931 strain than in that of 1923. It seems scarcely likely, therefore, that the control over rate of hormone concentration would be greater in the older line than in the newer one.

If this conclusion is justified, there is left the extent of the period of determination or of the range of stimulating concentrations of the hormone, or both, to account for intermediates. Nothing in the experiments here reported appears to provide a choice as between these two possibilities. However, embryonic development is known to consist in some animals, at least in part, of a chain of events in which some or all of the events provide the stimuli leading to later events. To whatever extent this is true, it would be difficult to imagine any great extension of the periods of determination of structures. Were many of these to be extended, and were each to wait upon the culmination of some preceding event, embryonic development would be correspondingly prolonged. Nothing is known that would render it probable that the embryos which become intermediate or mosaic adults take longer to develop, though the numerous ovarioles would make it possible for that to happen and for

the young aphids to be borne out of their regular turns based on the time of starting cleavage. On the whole, it would seem unlikely that embryonic development is retarded greatly by the factors leading to intermediacy.

The concentration of the hormone, on the contrary, is subject to no such known limitation. So far as anyone knows, the level of stimulation of completed differentiation could be farther above the level of beginning differentiation in one line of aphids than in another. So far as known there is no chain of events involved in the concentration of the hormone, such that one structure cannot begin to be stimulated to develop before the stimulation of another is completed.

It is suggested as more probable, therefore, that intermediacy in these aphids depends upon a gap between the thresholds of stimulation of beginning and of complete differentiation, rather than upon periods of determination or clustering of the rates of hormone development.

How this conclusion is related to the differences in the number of intermediate individuals induced by environmental conditions is difficult to ascertain. If the general concept that hormone concentration and range of stimulation levels combine to determine the winged type is correct, higher proportions of intermediates could be produced at the expense of winged aphids by lowering the hormone curves (reducing the rate of accumulation of the hormone) or at the expense of wingless aphids by elevating the hormone curves—all on the assumption, made to simplify the discussion, that the hormone is a stimulant rather than an inhibitor of the development of the characters of the winged females. The more striking changes in the number of intermediates produced by light and temperature in the 1923 strain were in general of a sign opposite to that of the change in the number of typical winged females produced by the same conditions. The suggestion contained in this comparison is that intermediates were being produced at the expense of winged adults. The reduction of the winged ones was in general, however, several times as great as the increase of the intermediates. In other words, many winged aphids were being converted into wingless ones as well as some into intermediates. This fact is not opposed to the supposition that hormone accumulation was being retarded; but since there were many wingless in most environmental conditions, a mere lowering of hormone curves would not necessarily leave any more of them in the intermediate zone. It would seem necessary to suppose that, in the lowering of the hormone concentrations, there was either an accumulation of the curves in the intermediate zone, or a widening of the ranges of stimulation so that more curves would intersect them.

One reason for not assuming an accumulation of hormone curves in the intermediate regions has already been pointed out.

It is worthy of note that in the 1931 strain, the only set of conditions which induced many intermediates served to increase, rather than diminish, the number of winged individuals (from wingless parents) or had practically no effect on the number of winged ones (from winged parents). If the explanations discussed above were to have general validity, it would be necessary to suppose that the intermediates from wingless parents were being produced at the expense of wingless ones, which might be due in part to a general lifting of the hormone curves. This contrast in the behavior of the two strains recalls their different responses to intermittent light in the production of wings; intermittent light produces fewer winged aphids in the 1923 strain, but more winged ones in the 1931 strain. There may be some common basis for these two differences. What reversal would be required in the hormone scheme to account for these contrasts it is impossible to say with any high degree of probability.

Whether intermediates are in any way dependent on the *direction* of some physiological change (that is, winged to wingless, or wingless to winged) can hardly be judged from the experimental methods of producing them. Analysis of the characters of the intermediates will be needed for an answer to this question.

SUMMARY

Two strains of aphids were subjected to various combinations of temperature and light. In one strain (collected 1923) distinctly more intermediate-winged females were produced in intermittent than in continuous light, in every combination of other conditions. Among those reared in intermittent light, more intermediates were produced at a temperature of 14° than at 24° , in every combination of other conditions. Those reared in continuous light, however, produced usually fewer intermediates at 14° than at 24° , and the differences were small. In general, winged parents produced more intermediate offspring than did wingless parents, but there was much irregularity of this relation. Greatest production of intermediates and greatest production of typical winged females did not occur at the same time; there was, indeed, a tendency for the two frequencies to be opposed to one another. Other relations in this strain were rather indefinite. In the second strain (collected 1931) more intermediates were usually produced in intermittent than in continuous light, but the differences were small. More intermediate offspring were produced by parents drawn from a stock kept at



room temperature than by parents taken from stocks kept continuously at 14° or 24°, or from a stock regularly alternated daily between 14° and 24°, though again most of the differences were small. The greatest (and the only large) number of intermediates in the 1931 strain were produced by taking aphids from a stock kept at 24°, changing them for one generation to room temperature, then putting this latter generation, when adult, at 14°. In general, in this strain the greatest frequency of intermediates occurred when the frequency of typical winged aphids was medium. Other relations in the 1931 strain were not striking.

If it be assumed that production of the characteristics of winged aphids be dependent on suitable concentrations of a hormone which increases in amount during development (other assumptions could be made), intermediacy or mosaicism might result from various combinations of times or periods of determination, or of levels or ranges of concentration of the stimulating hormone, or both. Ranges of stimulating concentrations are less subject to known limits than are periods of determination, and are held to be the more likely, inasmuch as strains of aphids differed greatly in their tendency to produce intermediates. Intermediacy, as contrasted with mosaicism, is favored by extended periods of determination and ranges of stimulation, especially if there is overlapping of these periods and ranges for the several structures.

In part, the intermediates may replace winged aphids in the 1923 strain, and wingless ones in the 1931 strains.

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